

CCXVIII. THE ANTISCORBUTIC VALUE OF FRESH LIME JUICE.

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STUDYING the antiscorbutic value of the lime, Chick, Hume and Skelton [1918] found that the majority of the preserved lime juices examined were valueless as a source of this important dietary factor. This valuable information satisfactorily explained the then growing lack of confidence in preserved lime juice as a protective or therapeutic agent against scurvy.

While determining the antiscorbutic content of preserved lime juice sent by the Department of Commerce and Industry, complete confirmation of the above observation was obtained. For none of the preserved juices subjected to the test contained any detectable quantity of the antiscorbutic principle.

With fresh lime juice, however, our results differed markedly from those reported by the same investigators. According to them, the minimum protective dose of fresh lime juice for a guinea-pig is 5 cc. daily, while in our case complete protection was attained with a much smaller dose. As a matter of fact our findings show clearly that there is no difference between the antiscorbutic content of the truly fresh ripe lime and that of the fresh lemon.

From the point of view of lime-growing countries like Egypt, this result is quite important both nutritionally and commercially. In this country fresh limes and lime juice are much preferred to lemons which are grown here on a limited scale. It follows that one may have to fall back on the lime in case of emergency, and it is, therefore, of great importance to know the exact place which must be assigned to this fruit among the antiscorbutic agents.

Before discussing the possible causes of the difference between our result and that observed by Chick and collaborators, a description of the experiments which led to this result will be given below.

Technique.

The diets. As a scurvy-producing diet the following ration was prepared: caseinogen 8, calcium carbonate 1.5, sodium chloride 1, oats 55, and bran to 100. This diet was given *ad lib.* supplemented with 0.5 cc. cod-liver oil daily per animal. When an adequate supply of the antiscorbutic was provided, the guinea-pigs grew at a good rate and appeared quite healthy. When, on the other hand, no antiscorbutic was given the animals exhibited, both during life

and at autopsy, a typical picture of scurvy. Death occurred 24 to 32 days from the commencement of the feeding and was always preceded by a decline in weight starting near the end of the second week.

For certain reasons a modification in the diet was introduced during the course of the experiment. The oats were replaced by an equivalent quantity of barley after proving that the change in no way influenced the scurvy-producing properties of the ration.

Experimental animals. Young guinea-pigs as obtained from a dealer were employed for the test. They were usually kept on greens and bread for 2 days before starting the feeding. Animals weighing 300–400 g. were, as a rule, selected, but in a very few cases guinea-pigs of weights slightly outside this range had to be used. The pigs were housed in capacious metal cages with wood shavings as bedding. Food and water were served *ad lib.* and the cod-liver oil and the juice under examination were fed by hand from a pipette.

It was found necessary to keep the mouth of the animals and the surrounding area of the face perfectly clean. When this was neglected stomatitis usually developed especially in those receiving a highly sugared juice. The food consumption of animals suffering from this disorder generally dropped, possibly from lack of appetite or from pain due to the inflamed condition of the mouth.

Preparation of the juice. The juice was prepared fresh daily from ripe fruits recently picked. Actually none of the fruits employed was older than 2 days at the outside. After being cut into halves, the limes were squeezed by hand into a muslin-covered funnel, and, to the strained juice, CaCO_3 and sugar were added in the proportion of 15 cc. juice, 0.5 g. CaCO_3 and 2 g. sugar and when all the sugar had dissolved, the volume was made up to 20 cc. with water. The mixture was then centrifuged and the supernatant fluid employed for the experiments.

The diagnosis of scurvy. A word may be said here about the plan adopted for the diagnosis of scurvy. It is intended to discuss only the *post mortem* findings. In severe cases, no difficulty is experienced in diagnosing the condition, for typical subcutaneous and intramuscular haemorrhages can be easily detected macroscopically in the characteristic sites. Similarly joint swellings, beading of the rib junctions, fragility of the long bones and looseness of the front teeth, which are constant accompaniments of the vascular changes, are also easily detectable. There are cases, however, which present a less complete picture of the disease. In these, haemorrhages are usually absent. Swellings of the joints and beading of the costochondral junctions, with or without fragile bones and loose teeth, are the only pathological changes to be seen. The diagnosis of these cases was not so easy, especially as similar bone changes have been ascribed to other deficiencies [Delf and Tozer, 1918]. Nevertheless these cases have been classed as mild scurvy, for they occurred only in animals receiving either an inadequate dose of the antiscorbutic or none at all. It will be remembered that each animal received by hand a daily dose of 0.5 cc. of tested cod-liver oil and that those getting an adequate supply

of the antiscorbutic principle were entirely free from any bone changes whatsoever. Another point, which seems to support the view that these bone changes are scorbutic in origin, is that in the same batch of guinea-pigs on the same insufficient dose of the antiscorbutic one encounters both types, that is, animals exhibiting at autopsy nothing but bone lesions and others showing haemorrhages as well.

The absence of scurvy was determined by the behaviour of the animals during life, especially as regards growth and general health, and by a careful study of the different systems after death.

A summary of the results obtained with fresh lime juice is embodied in Table I.

Table I. *Source of antiscorbutic principle. Fresh lime juice.*

Daily dose in cc.	Initial weight g.	Duration of exp. days	Result
0	250	25	Dead, scurvy
0	250	30	"
0	300	32	"
0	400	24	"
0.5	350	46	"
0.5	354	47	"
0.5	370	49	"
0.5	318	57	"
0.75	440	66	Dead, no haemorrhages, fragile bones, enlarged knee joints and rib junctions
0.75	338	104	Killed, chronic scurvy, knee joints ankylosed
1.0	333	51	Dead, moderate scurvy
1.0	300	62	"
1.0	340	93	Killed, mild scurvy, slight bone changes only
1.5	353	31	Dead, bronchopneumonia, no scurvy
1.5	358	93	Killed, no scurvy
1.5	343	44	Dead, liver abscess, no scurvy
1.5	320	93	Killed, no scurvy
1.5	350	93	"
2	270	93	"
2	300	93	"
3.5	380	93	"
3.5	250	93	"

From Table I above, it can be clearly seen that the minimum protective dose of fresh lime juice, obtained as described earlier, is 1.5 cc., and that partial protection is conferred even by 0.5 cc. which was the smallest dose tried.

Having established this point, it became interesting to define the relation of the Egyptian lime to that of the West Indies, since the latter fruit was employed in most of the previous work on this problem. There is no doubt that Egyptian limes belong to the group of the *Citrus medica* var. *acida* and, as a matter of fact, are, to the layman, indistinguishable from the West Indian limes, few trees of which are grown in the gardens of the Horticultural Section of the Ministry of Agriculture.

Many explanations can be suggested to account for the difference between the antiscorbutic value of fresh lime juice as reported here and that found by

other workers. But it appears that the difference is most probably due to the fact that, in previous work, the lime juice employed was obtained from relatively much older fruits and was sometimes kept for as long as 2 months before examination. If this explanation is true, the juice of West Indian limes examined under the conditions detailed above, ought to have an antiscorbutic value similar to that of fresh Egyptian limes. The results reported by Davey [1921] for fresh lime juice make this conclusion highly probable. With a special sample of lime juice, the minimum protective dose appeared to be less than that reported earlier by Chick *et al.* [1918]. This result also supports the view that, in lime juice, the antiscorbutic principle undergoes deterioration at a much quicker rate than in the case of other citrus fruits such as the lemon.

Owing to the close of the season, it was not possible to obtain direct information on this point. It was, however, decided to make some use of the few West Indian limes left on the trees, for comparison with Egyptian limes on the lines of the work done by previous investigators. West Indian limes grown locally and Egyptian limes were subjected to examination at one and the same time. In the preparation of the juice, quite fresh and ripe fruits were used. These were cut into two and squeezed by hand; and to the juice, strained through muslin, sodium benzoate was added as a preservative. The juice was kept in a refrigerator throughout the experiment and fed as such to the animals from a burette. The results obtained are given in Table II.

Table II.

Source of antiscorbutic	Initial weight g.	Duration of exp. days	Result
Egyptian Limes			
1 cc. daily	303	41	Died, bone changes only
"	358	42	Died, moderate scurvy
2 cc. daily	304	41	Died, bone changes only
"	315	40	"
3 cc. daily	350	45	"
"	270	51	Died, scurvy
West Indian Limes			
1 cc. daily	344	30	Died, moderate scurvy
"	304	36	"
2 cc. daily	360	35	"
"	364	56	Died, scurvy, severe
3 cc. daily	450	42	Died, moderate scurvy
"	432	40	"

Table II shows that the antiscorbutic principle undergoes deterioration in the juice obtained from Egyptian limes and kept in the refrigerator during the experiment, for 3 cc. failed to protect the animals against scurvy (*cf.* Table I). Compared with the West Indian lime juice, the animals on the Egyptian lime juice lived slightly longer. It is not possible, however, to attach any importance to this difference at present.

DISCUSSION AND CONCLUSIONS.

In determining the antiscorbutic value of fresh lime juice, Chick *et al.* [1918] employed imported limes which took 4 to 8 weeks in transit and the squeezed juice was fed for 2 months, that is, until a fresh consignment was received. Davey [1921], following a similar technique, arrived at practically the same conclusions regarding fresh lime juice.

In the first set of experiments presented above, the juice was prepared fresh daily from ripe fruits not more than 2 days gathered. If we agree that there is no essential difference between the lime grown in Egypt and that of other localities, the low antiscorbutic value reported previously for fresh lime juice can be ascribed to deterioration of the antiscorbutic principle. There seems to be no doubt that loss of this factor takes place at a much greater rate from lime juice than from lemon juice, and, as a result of the present work, one may conclude that loss starts in the intact fruit. Davey [1921] obtained slightly better results with green than with ripe limes. It thus appears that loss of the antiscorbutic principle commences with the process of ripening.

The experiments with the West Indian and Egyptian limes only show that in both juices the antiscorbutic principle is not stable for a long time. During the experimental period (approx. 2 months) deterioration was such that 3 cc. failed to protect against the disease.

SUMMARY.

1. The antiscorbutic value of lime juice, prepared fresh daily from ripe Egyptian limes not more than 2 days gathered, was found to be 1.5 cc. per guinea-pig daily.
2. 3 cc. of "fresh" lime juice, prepared under the same conditions but kept in the refrigerator during the whole course of the experiment (approx. 2 months), failed to protect the guinea-pig against scurvy.

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